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In re Application of:	)	Art Unit: 1647
EISENBACH-SCHWARTZ et al	)	Examiner: S. Turner
Appln. No.: 09/218,277	)	Washington, D.C.
Filed: December 22, 1998	)	May 11, 2001
For: T-CELLS, NERVOUS SYSTEM-SPECIFIC ANTIGENS AND THEIR USES	)	

REQUEST FOR PRIORITY

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with the provisions of 37 C.F.R. §1.55 and the requirements of 35 U.S.C. §119, there is filed herewith a certified copy of:

Appln. No.: 124550 Filed: May 19, 1998.

It is respectfully requested that applicant be granted the benefit of the priority date of the foreign application.

Respectfully submitted,

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חוק הפטנטים, תשכ"ז – 1967
PATENTS LAW, 5727-1967

בקשה לפטנט
Application For Patent

לשימוש הלשכה
For Office Use

מספר: Number	124550
תאריך: Date	19-05-1997
הוקדם/נדחה: Ante/Post-dated	

אני, (שם המבקש, מענו ולגבי גוף מאוגדת מקום התאגדותו)
I, (Name and address of applicant, and in case of body corporate-place of incorporation)

Yeda Research and Development Company Ltd. , Israeli Company, of P.O.Box 95, Rehovot 76100, Israel

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(באנגלית) (English)
Compositions comprising activated T-cells or T-cell activating agents and their use

מבקש בזאת כי ינתן לי עליה פטנט Hereby apply for a patent to be granted to me in respect thereof.

* בקשת חלוקה Application of Division	* בקשת פטנט מוסף Appl. for Patent of Addition	דרישת דין קדימה* Priority Claim		
מבקשת פטנט from application	* לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האיגוד Convention Country
No. מס'	No. מס'	121349	21.7.1997	Israel - IL
Dated מיום	dated מיום			
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המען למסירת מסמכים בישראל
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חתימת המבקש
Signature of Applicant

For the Applicants,
REINHOLD COHN AND PARTNERS
By : —

This **היום** 18 **בחודש** May **שנת** 1998
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תכשירים המכילים תאי T משופעלים או גורם המשפעל תאי T והשימוש בהם

Compositions comprising activated T-cells or T-cell activating agents and their use

ידע חברה למחקר ופתוח בע"מ

Yeda Research and Development Company Ltd.

C.109842.5

COMPOSITIONS COMPRISING ACTIVATED T-CELLS OR T-CELL ACTIVATING AGENTS AND THEIR USE

FIELD OF THE INVENTION

The present invention relates to compositions and methods for the treatment or diagnosis of damages in the central nervous system (CNS).

PRIOR ART

The following is a list of prior art which is believed to be relevant as a background to the present invention:

1. Stoll, G., Trapp, B.D., & Griffin, J.W., *J. Neurosci.*, **9**:2327-35 (1989).
2. Perry, V., & Gordon, S., *Int. Rev. Cytol*, **125**:203-44 (1991).
3. Perry, V. & Gordon, S., *Trends Neurosci.*, **11**273-277 (1988).
4. Jordan, F.L., Thomas, W.E. Brain macrophages: *Questions or origin and Interrelationships*, **13**:165-178 (1988).
5. Griffin, J.W., Stoll, G., Li, C.Y., Tyor, W. & Cornblath, D.R., *Ann. Neurol.*, **27**:8 (1990).
6. Giulian, D., Chen, J., Ingeman, J.E., George, J.K. & Noponen, M., *J. Neurosci.*, **9**:4416-29 (1989).
7. Giulian, D., *J. Neurosci. Res.*,**18**:155-171 (1987).

8. de Groot, C., Sminia, T. Dijkstra, C.D., *Immunobiology*, **179**:314-27 (1989).

9. Bauer, J., Sminia, T., Wouterlood, F.G. & Dijkstra, C.D., *J. Neurosci. Res.*, **38**:365-75 (1994).

10. Andersson, P.B., Perry, V.H. & Gordon, S., *Immunol. Lett.*, **30**:177-81 (1991).

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12. Perry, V.H., Brown, M.C. & Gordon, S., *J. Exp. Med.*, **165**:1218-1223 (1987).

13. Perry, V., Hume, D. & Gordon, S., *Neurosci.*, **15**:313-326 (1985).

14. Lunn, E.R., Brown, M.C. & Perry, V.H., *Neuroscience*, **35**:157-65 (1990).

15. Wekerle, H., *Curr. Opin. Neurobiol.*, **3**:779-784 (1993).

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17. Lazarov Spiegler, O., Solomon, A.S., Zeev Brann, A.B., Hirschberg, D.L., Lavie, V. and Schwartz, M., *FASEB J.* **10**:1296 (1996).

18. McIntosh, T.K., Novel pharmacologic therapies in the treatment of experimental traumatic brain injury: a review, *J. Neurotrauma* **10**:215 (1993).

19. Lynch, D.R., and Dawson, T.M., Secondary mechansims in neuronal trauma, *Curr. Opin. Neurol.*, **7**:510 (1994).

20. Smith, D.H., Casey, K, and McIntosh, T.K., *New Horiz.*, **3**:562 (1995).

21. Faden, A.I., *Pharmacol. Toxicol.*, **78**:12 (1996).

22. Faden, A.I., [editorial; comment], *JAMA*, **276**:569 (1996).

23. Popovitch, P.G., Stokes, B.T. and Whitecup, C.C., *J. Neurosci. Res.*, **45**:349 (1996).

These prior art references will be acknowledged herein by indicating their number from the above list.

BACKGROUND OF THE INVENTION

Traumatic injuries in the CNS are known to lead to an invasion of blood derived macrophages as well as activation of microglia within the brain [1-9]. The invasion is however delayed and more limited in its scope as compared to that in peripheral nerves traumas[2,10,13]. In addition, the duration of events associated with the acute phase of the injury is prolonged in the CNS as compared to the peripheral nervous system (PNS) and several weeks after injury, numerous activated macrophages and microglia are found in the CNS, while only a few are detectable in PNS nerves at such time after injury [12,14].

It is known that activated T-cells are able to cross the blood-brain barrier (BBB) and recognize epitopes in the CNS (Wekerle [15]), but the role of T-cells in enhancing or impeding CNS nerve regeneration, is not yet known.

Damage to the CNS may result from the physical injury from a disease, such as Alzheimer's disease (ED), etc. As is well known in the art, neurons in mammalian central nervous system (CNS) do not undergo spontaneous regeneration following an injury which affects their integrity. Thus, a CNS injury causes a permanent impairment in motor and sensory functions. This is in some contrast to neurons in the peripheral nervous system (PNS) where the axons have a far greater capacity to regenerate. PCT Application WO 97/09885[16] and Spiegler *et al.*, 1996[17] disclose a method and composition for the treatment of CNS injuries. In accordance with this PCT application, allogenic macrophages are incubated together with a stimulant (e.g. a nerve segment) and are subsequently administered into the CNS of a mammal at or near the site of injury. The method and compositions

disclosed in this PCT application give rise to at least partial regeneration of the impaired motor or sensory function.

One tragic consequence of CNS injury is that the primary damage is often compounded by a degenerative process which results in a secondary loss, over time, of adjacent neurons that were not damaged by the initial injury. It is believed that the secondary degeneration results from diffusion of toxic chemicals produced by damaged neurons[18-22]. It has recently been demonstrated that CNS trauma such as spinal injury triggers a systemic response to self epitopes such as myelin basic proteins (a BP)[23].

GENERAL DESCRIPTION OF THE INVENTION

The present invention is based on the finding that activated T-cells, regardless of the epitope recognized by them, accumulate at the site of CNS injury. It has been realized in accordance with the invention, that activated T-cells particularly such with a non-self epitope specificity may thus be used to advantage in delivering various substances to a damaged site in the CNS for various therapeutic and diagnostic applications. Furthermore, activated T-cells directed against self antigens which are manifested solely within the CNS were injected into an animal and were surprisingly found to inhibit secondary degeneration in the CNS. Based on these later findings, a variety of therapeutic approaches, all based on stimulation of autoimmune T-cells (ATCs) specific for CNS antigens, are provided, for the purpose of inhibition of or slowing down progression of CNS degeneration, following an initial CNS injury.

The present invention makes use, by some of its aspects with activated T-cells which are expanded *in vitro* and then inoculated into an individual with CNS damage. The T-cells are typically, and preferably, autologous. Such therapeutic T-cells are typically T-cells with specificity for antigens which are normally not found in the circulation or are present in the circulation in small

quantities. Such T-cells, once introduced into the circulation will not cause an autoimmune disease. These therapeutic T-cells may, for example, be T-cells with specificity for CNS-specific antigens, cryptic antigens or for non-self antigens. T-cells of the latter kind may for example be: T-cells with specificity for a different HLA or a different blood group type; T-cells with specificity for antigens of different organisms, e.g. T-cells with specificity for a bacterial antigen or a bacterial toxin, etc.

The use of such T-cells in therapy is novel and provided for the first time in accordance with the invention.

The invention also relates to the use of CNS-specific antigens, which are administered to individuals in order to induce *in vivo* proliferation of CNS-specific T-cells. A specific indication for which such CNS-specific antigen is used, is the treatment of CNS damages.

The present invention thus provides a method for the therapeutic treatment of an individual, comprising administering to the individual an effective amount of T-cells with specificity for antigens not present or present in the individual's circulation only in small quantities.

In accordance with the above method, T-cells are typically autologous T-cells withdrawn from the individual which are then exposed *in vitro* to said antigen for the purpose of expansion of T-cells with specificity for the antigen, and the expanded T-cell population is then inoculated to the individual.

Also provided by the invention is a pharmaceutical composition comprising, as an active ingredient, an effective amount of T-cells with specificity for an antigen not present or present in an individual's circulation only in small quantities. The pharmaceutical composition is typically such prepared for use in therapy of a specific individual with the T-cells being autologous T-cells.

The following are some terms which will be used in the description below, and their meanings in the context of this writing:

Effective amount – an amount of an active ingredient sufficient to achieve a therapeutic effect. For example, if the treatment involves a single administration of said active ingredient, an effective amount is an amount of this active ingredient sufficient to achieve a desired therapeutic effect. Where, by another example, the therapeutic treatment involves several consecutive administrations of said active ingredient, the effective amount may be an amount which although at times is insufficient to achieve a therapeutic effect by itself, yields a cumulative effect in several administrations, which ultimately achieves the desired therapeutic effect. The effective amount may depend on the intended therapeutic treatment, on the age or gender of the individual, on the severity of the treated condition, etc.

CNS damage/CNS injury - one of a variety of CNS injuries and genetic disorders which adversely affect the physiology of the CNS or impair neural pathways in the CNS. CNS damages or injuries include genetic degenerative diseases, e.g. Alzheimer's disease and Parkinson's disease; ischemias, i.e. loss of certain cells within the CNS as a result of impairment in blood flow, e.g. as a result of a stroke; various physical injuries, e.g. result of a strong impact on the skull such as a car accident, damages resulting from penetration of objects into the skull such as a bullet; inflammations within the CNS; etc.

Treatment of CNS damage/CNS injury – a treatment intended to improve or correct defects caused as a result of the damage and/or avoid further progress of the damage. For example, the treatment of CNS damage may be aimed in inducing regeneration of nerves within the CNS and/or avoid further axonal nerve cell degeneration.

Countering CNS degeneration - inhibition or slow down of progression of a degenerative process in the CNS. This may be a degenerative process resulting from a degenerative disease or disorder; secondary degeneration following a primary injury within the CNS; etc.

T-cells specific for "X"/T-cells with specificity for "X" – T-cells which recognize an antigenic epitope included in or on *"X"*. Exposure of such T-cells to antigen *"X"* results in their activities.

Activated T-cells - T-cells which were activated by an antigen to which they are specific and to which they will respond by proliferation.

Autoimmune T-cell (ATC) - an activated T-cell which has specificity towards a self antigen. This specificity is manifested, for example, by inducing proliferation of the T-cell, under appropriate condition, in the presence of the specific self antigen.

CNS–specific ATC - ATC which is specific for an antigen found exclusively within the CNS.

CNS-specific antigen – an antigen found exclusively within the CNS or an antigen which has an immune cross-reactivity with an antigen found exclusively within the CNS.

For the sake of convenience, the present invention will at times be specifically described with reference to different aspects thereof detailed below:

"Delivery Aspect" – by which activated T-cells are used to deliver substances to the CNS. The substances may either be substances carried and deliverable by the T-cells to the CNS or a substance which the activated T-cells can produce. Such a substance may be a substance of therapeutic or diagnostic utility. The substance may by one embodiment be a substance introduced into the T-cells *in vitro* by incubating the cells with such a substance. The substance, by another embodiment may be a substance produced by the cells, e.g. a substance naturally produced by stimulated T-cells, such as interleukin, growth factors, etc. or a substance produced by the T-cells following genetic manipulation of the cells, e.g. as a result of introducing an expressable DNA construct to the cells.

"Cell Therapy Aspect" – by which CNS-specific ATCs are used as active therapeutic agents. Preferred, in accordance with the invention is use of the CNS-specific ATCs for countering CNS degeneration.

"Immunization Aspect" – by which therapeutic use is made with CNS-specific antigens which are inoculated into an individual. Such inoculation may give rise, *inter alia*, to an increase in the number of CNS-specific ACTs. Preferred use of this active ingredient is in countering CNS damage.

Reference made to the above aspects, does not intend to limit the scope of the invention but merely to serve as a better illustration of the invention and the manner of carrying it out. It is thus clear that the invention thus applies to its full scope as defined above.

The use of CNS-specific ATCs or a CNS-specific antigen in human therapy, is novel and is provided for the first time in accordance with the invention.

The different therapeutic approaches in accordance with the invention may be applied independently or may be applied in combination. Particularly the Cell Therapy Aspect and the Immunization Aspect may be applied in combination to achieve increased protection against CNS-degenerative processes. Furthermore, these may at times be applied in combination with the Delivery Aspect, for example, the delivery of a regeneration inducing substances into the CNS and at the same time countering CNS degenerative processes.

In accordance with one embodiment of the invention there is provided a method for the delivery of a substance to a site of damage in an individual's central nervous system (CNS), comprising activating T-cells which either carry or can produce said substance, and allowing said cells to migrate within the individual's body to reach said site.

Also provided, by another embodiment is a composition for use in delivery of a needed substance to a site of CNS damage, comprising a pharmaceutically acceptable carrier and, as an active ingredient activated T-cells which either carry or can produce said substance.

Further provided, by an additional embodiment of the invention, are T-cells carrying or capable of producing a substance in need at a site of CNS damage, said substance being a substance which is not naturally carried or produced by the T-cells.

Still further provided, by another embodiment of the invention, is a method for diagnosis of damage in the CNS of an individual, comprising inoculating the individual with activated T-cells carrying a detectable marker substance, allowing time for migration of the T-cells to a site of damage in the CNS and then detecting the presence and location of the marker substance in the individual's CNS.

The invention also provides, by a further embodiment a method for the treatment of damage in an individual's CNS comprising inoculating the

individual with activated T-cells carrying or producing a substance in need at a site of CNS damage to ameliorate the damage or symptoms associated therewith.

The present invention further provides, by another embodiment, a pharmaceutical composition for the treatment of a human condition or disease, comprising a pharmaceutically acceptable carrier, and as an active ingredient,

i. CNS-specific ATCs; or

ii. CNS-specific antigen.

The present invention also provides, by a further embodiment, a therapeutic method for the treatment of a disease or condition in an individual, comprising administering to the individual an effective amount of CNS-specific ATCs or CNS-specific antigens.

Still further provided by an additional embodiment of the invention is use of CNS-specific ATCs as a CNS-specific antigen for the preparation of a pharmaceutical composition for the treatment of a human condition or disease.

As already noted above, a preferred use of the CNS-specific ATCs or the CNS-specific antigen, is for confering CNS degeneration.

DETAILED DESCRIPTION OF THE INVENTION

In the following the invention will be described in more details to be followed with a description of illustrative experimental results carried out in accordance with the invention.

Delivery Aspect

In accordance with this aspect activated T-cells, particularly, but not limited to such having a non-self epitope specificity may be used for the delivery of various substances to a damaged site within the CNS. Depending

on the nature of said substance the use of such T-cells may have utility in either therapy or diagnosis.

The activated T-cells carrying or capable of producing said substance may be inoculated immediately after their production or may be stored for later use.

The T-cells may be kept frozen under conditions so that after thawing, the cells become viable. The T-cells may also be formulated in a variety of compositions, e.g. in a medium which maintains viability of the T-cells for a time until they are inoculated into the body and which medium is also pharmaceutically acceptable for parenteral administration.

The T-cells may be activated by exposure of the cells to a variety of natural and synthetic antigens and epitopes, including super antigens such as LPS (lipopolysaccharide) which activate a large number of T-cell clones, or antigens which activate the number of T-cell clones, such as antigens used in the experiments described herein, and many others. During the *ex vivo* activation phase of the T-cells, a variety of growth promoting substances may be added to the T-cell containing medium including interleukins such as IL-2.

The T-cells are preferably syngeneic T-cells, particularly T-cells withdrawn from the same individual but they may also be T-cells obtained from HLA identical individuals. In addition, the T-cells may also at times be allogeneic T-cells, e.g. a pooled T-cell preparation obtained from a blood bank. The use of allogeneic T-cells is applicable particularly for a variety of one-time treatments, e.g., delivery of T-cells to a site of CNS damage for diagnostic purposes, for an acute one time therapy, etc.

The T-cells may be used for the delivery of a variety of substances to a damaged site of the CNS, including substances with a diagnostic utility, as well as substances having a therapeutic utility.

Detection of the presence and location of such T-cells may be by a variety of imaging techniques depending on the marker substance. For

example, if the T-cells carry metal particles, this may be achieved by magnetic resonance imaging (MRI) techniques; where the substance is a radioactive substance, the place of location may be achieved by a variety of imaging devices based on the detection of radioactivity.

For diagnostic application, T-cells will typically be loaded with contrasting agents, e.g., with metal such as gold particles, with radioactive markers, with gadolinium complexes (contrast agents for MRI), etc. The loading of the T-cells with such substance will be *ex vivo*, and after loading, the T-cells will be activated and then inoculated into the blood system, whereby at least a portion will localize at a site of damage in the CNS.

Loading of the T-cells with such substance may be achieved by a variety of means known *per se*. For example, T-cells may be incubated in a suspension comprising the metal particles and the loading will be a result of the spontaneous internalization of such particles into the cell's cytosol. Such substances may also be introduced into the cells by a variety of electrophoretic techniques. Loading of T-cells with a radioactive marker may be achieved by incubating cells with a radioactive metabolic precursor, e.g., for example, labeled with a radioactive marker having a short half life such as ^{131}Iodine and ^{99}Technecium, etc.

Substances with therapeutic utility include a variety of growth factors which promote nerve regeneration, substances lacking at the site of damage, e.g. a neurotransmitter, an anti-inflammatory substance, etc. Activated T-cells may spontaneously produce a variety of substances which may have a beneficial therapeutic effect on the damaged CNS site, including, for example, interleukins, growth factors and others. For therapeutic application, the T–cells may be treated *in vitro* to insert therein a DNA sequence which either encodes said substance, encodes an enzyme which catalyzes said substance, or encodes a regulatory product the production of which brings to expression of said substance in the T-cells, the DNA sequence being under

expression control such that it is either expressed continuously or induced to expression as a result of exposure of the T-cells to a microenvironment of a kind present at the site of DNA damage, typically to yield secretion of expression product of said DNA sequence. Examples are DNA sequences encoding the nerve growth factor (NGF), DNA sequences encoding enzymes which play a role in CNS nerve regeneration such as the enzyme transglutaminase, DNA sequences encoding an enzyme which catalyzes the production of a neurotransmitter, e.g. enzymes involved in the catalysis of acetylcholine or dopamine, etc. As a result, the T-cells which will localize at the site of damage in the CNS will produce and secrete the needed substances at the site.

The *"genetically engineered"* cells, i.e. the cells inserted with said DNA sequences, may be selected by a number of means, e.g. detecting production of said substance, use of a variety of expression markers, etc. The T-cells may both be stably transfected with said DNA sequences or may be transiently transfected. Transient transfection may at times suffice, particularly for acute one-time therapeutic regimes.

Therapeutic utilities include the induction of regeneration of damaged CNS nerves; delivery of missing neurotransmitters to damaged site at a variety of degenerative CNS disorders, such as delivery of acetylcholine into a degenerated site of the CNS in patients suffering from Alzheimer's disease, delivery of dopamine to degenerative sites in Parkinson's disease patients; etc.

The activated, substance-delivering T-cells, whether used for therapeutic or diagnostics, may be administered to the individual by intravenous or intraperitoneal injection.

Cell Therapy and Immunization Aspects

Said human condition or disease treatable in accordance with these aspects, is typically, but not exclusively, CNS degeneration. Particularly, the

active ingredients of these aspects are useful in countering CNS degenerative processes. For example, said active ingredients are useful in a treatment intended to counter secondary degeneration which may otherwise follow primary CNS injury, e.g. a cut or a crush in a CNS tissue. In addition, said active ingredients may also be used to counter various other degenerative processes, e.g. a degeneration which may occur in either grey or white matter (or both) as a result of various diseases or disorders, for example senile dementias, Alzheimer's disease, Parkinson, Glaucoma, prior diseases such as Mad-Cow disease, etc.

The therapeutic treatment may be a short term or long term treatment, depending on the condition or disease which is being treated. In the case of a CNS injury, the treatment may be for a period of time ranging between several days to months or even years, until the condition has stabilized and there is no or only a limited manageable risk of development of secondary degeneration. In chronic human diseases or conditions such as Alzheimer's disease or Parkinson's, the therapeutic treatment in accordance with the invention may at times be for life.

At times, it is sufficient for said active ingredient to be administered once in the course of therapeutic treatment. Occasionally, however, the administration may have to be repeated periodically, e.g. once a month, once in three months or six months, etc.

As will also be appreciated, the therapeutic effect depends at times on the condition or disease to be treated, on the individual's age and health condition, on other physical parameters (e.g. gender, weight, etc.) of the individual, as well as on various other factors, e.g. whether the individual is taking other drugs, etc.

The composition of the invention is typically a parenteral composition, for intravenous (i.v.) or intraperitoneal (i.p.) administration. In the case said

active ingredient is a CNS-specific antigen, it may at times also be possible to formulate and utilize the composition for intramuscular (i.m.) administration.

In accordance with one embodiment, said active ingredients are given to an individual in combination with a therapeutic treatment intended to promote regeneration of axons within the CNS, the latter therapeutic treatment being, for example, a treatment according to the Delivery Aspect where said substance has an effect in promoting nerve regeneration; or that described in PCT Application No. WO 97/09985[(1)], the disclosure of which is incorporated herein by reference. Briefly, in accordance with this PCT application, alogeneic mononuclear phagocytes are used to promote axonal regeneration in the central nervous system of a mammal. The mononuclear phagocytes which are typically cultured *ex vivo* together with a stimulating tissue, such as dermis or a nerve segment, are administered into the central nervous system of a mammal at or near the site of injury or disease-inflicted lesion. The mononuclear phagocytes of this PCT application are preferably autologous. Typically, in such a combination therapy (namely a combined administration of mononuclear phagocyte cells according to PCT Application No. WO 97/09985 and of CNS-specific ATCs, in acordance with the present invention), the treatment will involve both injection of the mononuclear phagocytes into the site of injury or lesion within the CNS, and either at the same time, prior thereto or thereafter, the CNS-specific ATCs or CNS-specific antigen, will be administered parenterally to the individual.

These aspects of the invention will now be further illustrated below in more details with specific emphasis on the use of the active ingredients of the invention in countering degenerative processes in the CNS. It should be appreciated, that although this is a preferred embodiment of the invention, the invention is not limited thereto.

CNS-specific ATCs

In accordance with the cell therapy aspect of the invention, use is made of CNS-specific ATCs. A preferred embodiment of the invention is the use for countering CNS degeneration, particularly in inhibiting progress of CNS degeneration.

Circulating T-cells of healthy humans, which are known to contain such which act specifically against myeline basic protein or other CNS antigens such as the amyloid precursor protein, will be isolated and expanded using known procedures. In order to obtain CNS-specific ACTs, blood is withdrawn, T-cells are isolated and the CNS-specific ATCs are then expanded by known procedures (Burns *et al., Cell Immunol.* **81**:435 (1983); Pette *et al., Proc. Natl. Acad.* Sci. USA **87**:7968 (1990); Mortin *et al., J. Immunol.* **145**:540 (1990); Schluesener *et al., J. Immunol.* **135**:3128 (1985); Suruhan-Dires Keneli *et al., Euro. J. Immunol.* **23**:530 (1993).

Following their proliferation *in vitro*, the T-cells are injected into the patients. T-cell expansion is typically performed using peptides corresponding to sequences in a self CNS protein that are non-pathogenic, with the aim of obtaining the maximal benefit of autoimmunity without risk of autoimmune disease.

An individual can initially be immunized with a CNS-specific antigen using a non-pathogenic peptide of the self protein. This procedure does not carry the risk of autoimmune disease. From the blood of these immunized animals a T-cell preparation will be prepared, preferably from T-cells selected for their specificity towards the CNS-specific antigen. The selected T cells can then be stimulated to produce a T-cell line specific to the self-antigen (Ben Nun *et al., J. Immunol.* **129**:303 (1982)).

The CNS-specific antigen may be a pure antigen, a crude CNS preparation, a peptide derived from a CNS-antigen, as will be described below (see also *"CNS-specific antigen"*).

The CNS-specific ATCs, obtained as described above, can be used immediately or may be preserved for later use, e.g. by cryo preservation. The T-cells may also be preserved for later use, prior to a final stimulation stage; in other words, after revitalizing the cells following the preservation, e.g. thawing following the cryopreservation, the T-cells may be incubated with CNS-antigen, possibly together with thymocytes, for the purpose of stimulation so as to obtain a preparation of CNS-specific ATCs.

CNS-specific antigen

The CNS-specific antigen is used as an active ingredient, in accordance with the immunization aspect of the invention. It may typically be administered to the individual parenterally, which may include all types of parenteral administration modes, particularly intramuscular, intraperitoneal, subcutaneous, and intravenous. Furthermore, the CNS-specific antigen may also be used as a stimulant for the CNS-specific ATCs.

The CNS-specific antigen may be a purified antigen obtained from CNS tissue, particularly such tissue found at a site of CNS injury. In addition to a purified antigen, the CNS-specific antigen may also be a derivative of such an antigen, e.g. a peptide derived from an antigenic protein, as well as any other agent which can elicit an immune reaction towards CNS-specific antigens. Particularly preferred are peptides derived from self CNS proteins, but when injected, unlike the whole protein, although activating T-cells, do not induce an autoimmune disease. An example of such a peptide is a peptide comprising amino acids 51-70 of myelin basic protein.

In addition, the CNS-specific antigen may be crude CNS-tissue preparation, e.g. derived from tissue obtained at the site of CNS injury. Such a preparation may include cells, both living and dead cells, membrane fractions of such cells or tissue, antigens which have been obtained by genetic engineering methods, etc. Generally, the CNS-specific antigens include all

antigens which can elicit an immune reaction toward antigens present exclusively within the CNS.

The CNS-specific antigen may be obtained by a CNS biopsy from an individual, particularly a biopsy from a site of CNS injury, may be obtained from cadavers, may be obtained from cell lines grown in culture, may be a protein obtained by genetic engineering, etc.

The CNS-specific antigen may be kept in solution, may be provided in a dry form, e.g. as a powder or lyophilizate, to be mixed with appropriate solution, cell, prior to use.

Formulation and administration

(a) Activated T-cells or CNS-specific ATCs

For administration, the activated T-cells or CNS-specific ATCs are suspended in a sterile, pharmaceutically acceptable carrier, and inoculated parenterally, particularly i.p. or i.v. The pharmaceutically acceptable carrier may, for example, be a saline solution, such as a phosphate buffered saline (PBS), or a physiologically compatible culture medium. Generally, any carrier which is physiologically compatible and compatible with the cells, can be utilized.

(b) CNS-specific antigen

A CNS-specific antigen is administered to an individual in a sterile pharmaceutically acceptable carrier. The carriers may typically be saline, such as PBS, or any other physiologically compatible medium.

At times, in order to obtain an improved immune response, the CNS-specific antigen may be administered together with an adjuvant, such as Incomplete Freund's Adjuvant (IFA) and many others.

(c) Therapeutic Regiment

The activated T-cells according to the Delivery Aspect of the invention, which deliver a therapeutic substance, the CNS-specific ATCs, in accordance with the cell therapy aspect of the invention, as well as the CNS-specific antigen, in accordance with the immunization aspect of the invention, are preferably given to the individual shortly after injury or detection of a degenerative lesion in the CNS. It is generally believed that it is advantageous to treat the individual so as to induce regeneration or to counter progression of degenerative processes in the CNS as early as practically possible. The therapy may involve inoculation of only activated T-cells, CNS-specific ATCs, or only CNS-specific antigens, or a combination of these, e.g. both CNS-specific ATCs and CNS-specific antigens. The treatment may involve a single inoculation, or may involve a plurality of inoculations of either the CNS-specific ATCs, the CNS-specific antigen, or both, periodically over a period of time, e.g. once a month, once a quarter, once every six months, etc., during the course of treatment, which may last several months, several years or occasionally also through the life time of the individual.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a montage of epifluorescence micrographs of serial sections of an optic nerve excised fourteen days after a controlled crush injury. At the time of crush, the tested animals were injected intraperitoneally [ip] with $5x10^5$ T-cells primed to the antigen myelin basic protein (MBP) (T_{MBP}) that were labeled with Hoechst stain. One section was stained with anti GFAP (antibodies against glial fibrillary acidic protein) to visualize the injury site (lack of GFAP positive astrocytes) and photographed using filters that detect rhodamine and the others were photographed using filters that

detect Hoechst stain to visualize the labeled cells (these appear as white dots). The figure shows that injected T_{MBP} cells selectively accumulate at the injury site (I) and just distal (D) to the injury.

Fig. 2 shows a higher magnification of the site of optic nerve injury shown in Fig. 1, showing the large concentration of injected cells localized in the site of injury.

Fig. 3 shows a serial section through non-injured optic nerve.

Fig. 4 is a graphical representation of the number of T-cells, of two different T-cell clones primed with antigens MBP and ovalbumin (OVA) (T_{MBP} and T_{OVA}) after injury, at various time intervals following injury. T_{MBP} and T_{OVA} cells were injected into animals at the time of optic nerve crush, ipsolateral and contralateral nerves were then removed and prepared for microscopy at days 3, 7, 14 and 21. The figure shows that T-cells accumulated at the site of injury, independent on the antigen they were primed with (each result is an average of 5 different experiments; the bar shows the standard deviation).

Fig. 5 shows accumulation of T-cells measured immunochemically using antibodies to T-cell receptors. The results show a comparison of the number of accumulated cells in injured optic nerve (ON) and in non-injured Optic nerve (*"T_{MBP}"* and *"T_{OVA}"* - as above).

Fig. 6 shows accumulation of T-cells in injured and non-injured optic nerve after various treatment protocols. T-cells specific to MBP (T_{MBP}) were injected either immediately after nerve injury (T_{MBP} Cell Injection = 0) or 14 days after injury (T_{MBP} Cell Injection = 14) and their accumulation at the optic nerve was analyzed either 7 days (nerve excision - day 7) or 21 days (nerve excision = day 21) after injury.

Fig. 7 shows the T-cell accumulation in injured optic nerve 1 week after injury. Anti-MBP or anti-OVA or anti-hsp60 T cell lines were raised, maintained, and activated by incubation with MBP from the spinal cords of

guinea pigs, or with OVA (Sigma), or with the 51 to 70 peptide of MBP, respectively, in the presence of irradiated (2000 rad) syngeneic thymus cells, as described (Burns, *et al., supra* (1983)). Activated T-cells ($1x10^7$ cells) of the anti-MBP or anti-OVA lines or PBS were injected intraperitoneally into adult Lewis rats immediately after unilateral crush injury of the optic nerve. Seven days after injury both optic nerves were removed, cryosectioned, and analyzed immunohistochemically for the presence of labeled T-cells. Note the striking increase in T-cell accumulation in injured nerves compared to uninjured nerves of rats injected with all T-cells. Bars shows the mean total numbers of T-cells counted in 2 or 3 sections of each nerve. Each group contained 3 or 4 rats. ANOVA disclosed significant differences in T-cell numbers between 9a) injured optic nerves of rats injected with anti-MBP T-cells and injured optic nerves of rats injected with PBS ($p<0.001$); (b) injured optic nerves of rats injected with anti-OVA T-cells and injured optic nerves of rats injected with PBS ($p<0.05$); (c) injured optic nerves and uninjured optic nerves of rats injected with anti-MBP T-cells ($p<0.001$); (d) injured optic nerves and uninjured optic nerves of rats injected with anti-OVA T cells ($p<0.01$); and (e) injured optic nerves and uninjured optic nerves of rats injected with PBS ($p<0.05$).

Fig. 8 illustrates that T-cells specific to MBP, but not to OVA or hsp60, protect neurons from secondary degeneration after partial optic nerve crush injury in adult rats. The effects of the injected T-cells on the numbers of surviving optic nerve fibers were monitored by retrograde labeling of the retinal ganglion cells (RGCs) following application of 4-Di-10-Asp distally to the site of the primary lesion, immediately after injury (for assessment of primary degeneration) and 2 weeks later (for assessment of secondary degeneration). Uninjured optic nerves were similarly labeled at approximately the same distance from the globe. Five days after dye application, with the animals under deep anesthesia, the retinas were excised and flat-mounted in

4% paraformaldehyde solution, and labeled RGCs were counted under the fluorescence microscope. Counting was done in 5 randomly selected fields in each retina (all located at approximately the same distance from the optic disc). using this labeling approach, only those RCGs whose axons are still viable can be labeled. The numbers of RCGs in each group of injured nerves in rats injected with PBS only or injected with anti-MBP, anti-OVA, or anti-hsp60 T-cells were expressed as percentages of the total numbers of spared neurons following the primary injury (42% of the axons remained viable after the primary injury). The protective effect of the anti-MBP T-cells was significant ($p<0.001$, ANOVA). The anti-OVA T-cells and the anti-hsp60 T-cells did not differ significantly from PBS injection in their effect on the protection of nerves that had escaped the primary injury ($p>0.05$, ANOVA).

Fig. 9 show photomicrographs of retrogradable labeled retinas of injured optic nerves of rats injected with PBS, anti-hsp60 T-cells, or anti-MBP T-cells. Immediately after crush injury of the optic nerves, animals were injected i.p. with PBS or $1x10^7$ activated anti-hsp60 T-cells or $1x10^7$ activated anti-MBP T-cells. Two weeks later, 4-Di-10-Asp was applied to the nerves distally to the site of injury. After 5 days the retinas were excised and flat-mounted. Labeled RGCs located at approximately the same distance from the optic disk in each retina were photographed. The figure shows surviving RGCs in (A) injured optic nerves of rats injected with PBS, (B) injured optic nerves of rats injected with anti-hsp60 T-cells, and (C) injured optic nerves of rats injected with anti-MBP T cells.

Fig. 10 demonstrates that the neuroprotective effect of anti-MBP T-cells is not related to autoimmune disease. Two T-cells lines specific to MBP were used in this experiment. A substantial degree of disease (EAE) was evoked by one line and very weak disease by the other. The effects of the injected T-cells on the numbers of surviving optic nerve fibers were monitored by retrograde labeling of the retinal ganglion cells following 2

applications of 4-Di-10-Asp distally to the site of the primary lesion, the first immediately after injury (for assessment of primary degeneration) and the second 2 weeks later (for assessment of secondary degeneration). Note that both lines had a neuroprotective effect on secondary degeneration compared to that seem in control crush-injured but non-injected rats.

Fig. 11 shows that the clinical severity of EAE is not influenced by optic nerve crush injury. Rats were injected i.p. with $1x10^7$ activated anti-MBP T-cells either immediately after optic nerve crush injury or without optic nerve crush injury. The clinical course of rats injected with anti-MBP T-cells was evaluated according to the neurological paralysis scale. Each group contained 5 to 9 rats. Data points represents means ± SEM.

Fig. 12 shows the survival of neurons, inspected by rescue RGCs in uninjured nerves is not influenced by injection of anti-MBP T-cells. Lewis rats were injected i.p. with $1x10^7$ activated anti-MBP T-cells or PBS. Two weeks later, 4-Di-10-Asp was applied to the optic nerves. After 5 days the retinas were excised and flat-mounted. Labeled RGCs from 5 fields (located at approximately the same distance from the optic disk) in each retina were counted and their average number per mm^2 was calculated. There was no difference in the number of surviving neurons measured by labeled RGCs between rats injected with anti-MBP T-cells and control (PBS-injected) rats.

EXPERIMENTS

1. Materials and Methods

1.1 Animals

Female Lewis rats were obtained from Harlan Olac (Bicester, UK), matched for age (8-12 weeks) and housed four to a cage in a light and temperature-controlled room.

1.2 Proteins used for T-cell stimulation

Myelin basic protein (MBP) was prepared from guinea pig spinal cord as previously described (Ben-Nun *et al., supra* (1982)). Chick ovalbumin (OVA) was purchased from Sigma (Israel). Heat-inactivated *Mycobacterium tuberculosis* H37RA (*M. tuberculosis*) and Incomplete Freund's adjuvant (IFA) were purchased from Difco Laboratories (Detroit, MI, USA).

1.3 Media

The proliferation medium of the T-cells contained the following: Dulbecco's modified Eagle's medium (DMEM, Biological Industries, Israel) supplemented with 2mM L-glutamine (L-Glu, Sigma, USA), $5x10^{-5}$M 2-mercaptoethanol (2-ME, Sigma), penicillin (100 IU/ml; Biological Industries), streptomycin (100 μg/ml; Biological Industries), sodium pyruvate (1 mM; Biological Industries), non-essential amino acids (1 ml/100 ml; Biological Industries) and autologous rat serum 1% (vol/vol) (Mor *et al.*, *Clin. Invest.*, **85**:1594 (1990)). Propagation medium contained: DMEM, 2-ME, L-Glu, sodium pyruvate, non-essential amino acids and antibiotics in the same concentration as above and also 10% fetal calf serum (FCS), and 10% T-cell growth factor (TCGF) obtained from the supernatant of concanavalin A-stimulated spleen cells (Mor *et al., supra*, 1990).

1.4 Establishment of T-cell lines with active EAE (experimental allergic encephalomyelitis) induction

MBP and OVA were dissolved in PBS (1 mg/ml) and emulsified with an equal volume of IFA supplemented with 4 mg/ml *M. tuberculosis*. Rats were immunized subcutaneously in the hind footpads with 0.1 ml of the emulsion. At day 9 (1-3 days before

clinical onset of disease), animals were euthanized and draining lympnodes were surgically removed and dissociated under sterile conditions. The cells were washed and placed in stimulation medium with irradiated thymocytes (2000 rds) and either 10 μg/ml of MBP, OVA or *M. tuberculosis* for 3 days. Cells were then washed and placed in propagation medium for 5 to 10 days at which time they were restimulated again with irradiated thymocytes and peptides in proliferation medium. T-cell lines were expanded by stimulation and propagation and tested for specificity in an antigen specific T cell proliferation assay. Lines were expanded and stocks were frozen in liquid nitrogen. For experiments the cells were thawed and stimulated once before being used in experiments.

1.5 Passively transfer of labeled and non-labeled T-cell lines

T-cell lines were activated by restimulation *in vitro* with their own antigen (10 μg/ml) as above in proliferation medium. After incubation for 48-72 hrs at 37°Cm 90% relative humidity and 7.5% CO_2, the cells were washed and viable cells were isolated on Percoll and suspended in PBS. Animals were injected with 10 x 10^6 cells/ml i.p., and control rats were injected with 1 ml PBS i.p.

1.6 Crush injury of rat optic nerve

Crush injuries were performed as previously described (Hirschberg *et al.*, 1994). Briefly, rats were deeply anesthetized by i.p. injection of xylazine (10 mg/kg; Rompun) and ketamine (50 mg/kg; Velalar). Under a binocular operating microscope, a lateral canthotomy was performed in the right eye and the conjunctiva was incised lateral to the cornea. After separation of the retractor bulbi muscles, the optic nerve was exposed intraorbitally by blunt dissection. A moderate crush

injury was inflicted on the optic nerve, 2 mm from the eye, using a calibrated cross-action forceps (Duvdevani *et al., Instructure Neurology and Neuroscience*, 2:31, 1990). The contralateral nerve was left undisturbed and was used as a control.

1.7 Crush injury of rat sciatic nerve

Under deep anesthesia as described above, the sciatic nerve was exposed and a similar crush injury was inflicted. At the end of the operation the skin was sutured.

1.8 Sectioning of nerves

At specified time points, rats were euthanized by over-anesthesia with ether and their optic nerves were surgically removed, immersed in Tissue-Tek (Miles Inc., USA), and frozen in liquid nitrogen cooled in iso-pentane (BDH, UK). The nerves were then transferred to dry ice and stored at -70°C until sectioning. Longitudinal cryostat nerve sections (20 μm thick) were picked up on to gelatin-coated glass slides (four sections per slide) and frozen at -20°C until viewed or prepared for fluorescence staining.

1.9. Data analysis of T-cells in nerve sections

Nerves excised at various time periods after injury were prepared and sectioned. Hoechst-labeled nuclei or immunostained cells in each section were counted using the fluorescence microscope. For each time point five sections were counted, and the numbers were averaged.

1.10 Immunolabeling of nerve sections

Longitudinal cryostat nerve sections (20 μm thick) were picked up onto gelatin glass slides and frozen until preparation for fluorescent staining. Sections were thawed and fixed in ethanol for 10 minutes at room temperature, washed twice with double-distilled water (ddH_2O), and incubated for 3 minutes in PBS containing 0.05% polyoxyethylene-sorbitan monolaurate (Tween-20; Sigma, USA). They were then incubated overnight at 4°C with a mouse monoclonal antibody directed against rat macrophages (ED1; 1:400; Serotec, UK) and antibody against rat glial fibrillary acidic protein (GFAP; 1:100; BioMakor), all diluted in PBS containing 3% FCS. Staining of T-cells was accomplished by incubating nerve sections for 1 hr at room temperature with a mouse monoclonal antibody directed against rat T-cell receptor (TCR) (1:100, Hunig *et al., J. Exp. Med.*, **169**:73, 1989), in PBS containing 3% FCS and 2% BSA. After three washes with PBS containing 0.05% Tween-20, the sections were incubated with goat anti-mouse $F(ab')_2$ conjugated to either fluorescein isothiocyanate (FITC; BioMakor) or tetramethyl rhodamine isothiocyanate (TRITC; BioMakor) at a dilution of 1:100 and 1:50 respectively, for 1 hr at room temperature. They were then washed with PBS containing Tween-20 and treated with glycerol containing 1,4-diazobicyclo-(2,2,2) octane (Sigma), to inhibit quenching of fluorescence. The sections were viewed with a Zeiss Universal fluorescence microscope using filters that detect TRITC, FITC and Hoechst stains (Blaugrund *et al., Exp. Neurol.*, **118**:105, 1992, Blaugrund *et al., Brain Res.*, **574**:244, 1992).

1.11 Retrograde labeling of RGCs

The optic nerve was exposed, without damaging the retinal blood supply. Solid crystals of the dye-4-(4-(didecylamino)styryl)-n-methyl-pyridinium iodide (4-Di-10-Asp) (Molecular Probes, Europe BV) were deposited 1-2 mm from the distal border of the injury site. Non-injured optic nerves were similarly labeled at approximately the same distance from the globe. Five days after dye application, the retinas were excised under deep anesthesia, flat mounted in 4% paraformaldehyde solution, and labeled RGCs were counted by fluorescence microscopy.

1.12 Assessing the effects of injected T-cells on the numbers of surviving optic nerve fibers

The effect of injected T-cells on the numbers of surviving optic nerve fibers was monitored by retrograde labeling of RGCs (see above) immediately after injury (in order to assess primary degeneration) and two weeks later in order to assess secondary degeneration. Five days after dye, (4-Di-10-Asp) application the retinas were excised, whole mounted and their RGCs were counted. The counting was done in five randomly selected fields in each retina (all located at approximately the same distance from the optic disc) in all cases the dye was applied 2 ml distally to the site of the prior insert. Using this lengthening approach, only those RGCs whose axons were still viable could be labeled. The numbers of RGCs in each group of injured nerves treated with PBS only were injected with T_{MBP} or T_{OVA} cells were expressed as percentage of axons, out of those which survived the primary insult (42% the axons remained after the primary insult).

1.13 Clinical evaluation of EAE

Clinical disease was scored every 1 to 2 days according to the following neurological scale: 0, no abnormality; 1, tail atony; 2, hind limb paralysis; 3, paralysis extending to thoracic spine; 4, front limb paralysis; 5, moribound state.

2. Results

2.1 Activated T-cells accumulated at a site of CNS damage

T-cell clones primed to MBP (T_{MBP}) were stimulated with MBP for 2 days before being labeled with Hoechst stain and injected into animals ip at the time of injury. At 3, 7, 14 and 21 days after injury, the nerves were excised, cryosectioned and analyzed microscopically for the presence of labeled T-cells. T_{MBP} cells were detected in the injured optic nerves at day 3 and accumulated until a peak at day 14 (Fig. 1). Large clusters of T_{MBP} cells were observed at the injury site and fewer individual cells were seen proximal and distal (Fig. 2). Four weeks after injury, labeled T-cells were still detectable in the degenerating optic nerves. No T-cells were found in the non-injured optic nerves (Fig. 3), non-injured sciatic nerve or injured sciatic nerve at any time after injury. Labeled T-cells were occasionally found in capillaries and in connective tissue but were not concentrated or localized into any specific areas. T-cells that were not prestimulated with antigen did not accumulate in any of the nerves, including damaged nerves.

The accumulation of T_{MBP} cells in injured CNS, but not in injured PNS, suggests that there might be some specific interaction between the primed T cells and the CNS tissue from which the MBP antigen was originally derived. To determine whether the injured CNS interacted with T-cells in general, or specifically with T-cells primed with a CNS antigen, the previous experiments were repeated using a clone that responds to chick ovalbumin (T_{OVA}). Rats were injected with a labeled T_{OVA} clone pre-

stimulated with ovalbumin (OVA) using the same protocol as with the T_{MBP} cells. The labeled T_{OVA} cells accumulated in injured optic nerve, and the pattern of accumulation was similar to that of the T_{MBP} cells. Labeled T_{OVA} and T_{MBP} cells were counted in longitudinal sections of optic nerve prepared, 3, 7, 14 and 21 days after injury. No significant different was observed in numbers of T_{MBP} and T_{OVA} cells in injured optic nerve (Fig. 4), indicating that antigen specificity has little to do with the accumulation of T-cells in CNS injury sites. T_{MBP} cells were detectable slightly earlier than T_{OVA} cells in the optic nerve injury site, and antigen specificity may play a role in this but is not sufficient to explain the large accumulation of T_{OVA} cells in the site of injury.

Fig. 5 shows accumulation of T-cells measured immunocytochemically using antibodies to T-cell receptors. This detection technique rules out the possibility that the observed labeling is due to phagocytic cells which had phagocytized the pre-labeled T-cells shown in Fig. 1. The graph shows a striking elevation in T-cell accumulation following injury, regardless of whether the systematically injected T-cells are specific to a self-epitope (MBP) or to a non-self epitope (OVA).

Fig. 6 shows that accumulation of T-cells is dependent on the lesion and not the breakdown of the blood-brain barrier. T-cells specific to either MBP or OVA were injected 2 weeks after injury and their accumulation analyzed a week later, namely 21 days following the primary lesion. Their accumulation was compared to that of T-cells injected immediately after injury and detected either 7 or 21 days later. It appears that the time elapsed between the injury and the injection of T-cells, which is a factor in the sealing of the blood-brain barrier, is not a factor in the T-cell accumulation.

2.1 Accumulation of CNS-specific ATCs

The injured optic nerve was analyzed for T-cell accumulation. As shown in Fig. 7, in the uninjured optic nerves of control rats

injected with phosphate-buffered saline (PBS) no T-cells could be detected. Small but significant numbers of T-cells were observed in the uninjured optic nerves of rats injected with anti-MBP T-cells (primed against a peptide comprising amino acids 51-70 of MBP *"P51-70"* known to be capable of inducing experimental autoimmune encephalomyelitis (EAE) under these experimental conditions), but not of rats injected with anti-OVA T-cells. Crush injury of the optic nerve was accompanied by a small but significant accumulation of endogenous T-cells, possibly reflecting a response to self antigens triggered by the injury. In the injured optic nerves, T-cell accumulation was significantly increased by 5- to 6- fold) in rats injected with anti-OVA, anti-hsp60, or anti0MBP T-cells. These observations confirmed our previous finding that axonal injury in the CNS is accompanied by the accumulation of endogenous T cells and that this accumulation is augmented by systemic injection of activated T-cells irrespective of their antigenic specificity.

2.2 Protection of secondary nerve degeneration by T-cells specific to MBP

The course of secondary degeneration as a result of the injected T-cells was then examined. previous studies have shown that a time lapse of 2 weeks between a crush injury (of similar severity to the present one) and dye application is optimal for demonstrating differences (in terms of the numbers of still-viable, i.e. labeled, neurons) in degeneration with and without neuroprotection. As shown in Fig. 8, in retinas of injured nerves that were subjected to dye application 2 weeks after injury and excised a week later, the number of labeled ganglion cells (reflecting still-viable axons) was about 2.5- fold greater in animals injected at the time of injury, with T-cells specific to MBP (primed against P51-70 than with PBS. In

contrast, labeled ganglion cells in the retinas of rats injected with anti-OVA or anti-hsp60 T-cells were not significantly more numerous than in the retinas of rats injected with PBS. Fig. 9 represents micrographs of regrogradeably labeled retinas of injured optic nerves of rats injected with PBS, anti-hsp60 T-cells, or anti-MBP T-cells.

Since only the anti-MBP T-cells showed a neuroprotective effect, and as hsp60, like MBP, is a self antigen which is expressed in injured tissues including EAE lesions, it was interesting to find out whether the protective effect of the anti-MBP T-cells is *a function* of their *aggressiveness in causing* an autoimmune disease. If so, this would explain the lack of a protective effect by hsp60, which is also a self antigen, but, unlike MBP, is not restricted to the CNS and the T-cells specific to it do not cause a disease. To explore the possibility of a connection between the observed neuroprotective effect and autoimmune disease, the effect of T-cells generated against an epitope in MBP (P51-70) that does not cause an autoimmune disease was examined. As shown in Fig. 10, the neuroprotective effect of these non-aggressive anti-MBP T-cells was similar to that of the anti-MBP T-cells that cause autoimmune diseases. It thus seems that the observed beneficial effect of the T-cells on secondary degeneration is not common to all self antigens, but in this study is restricted to MBP (and possibly to other myelin-associated antigens as well), and furthermore that the connection is not between neuroprotection and autoimmune disease but between neuroprotection and autoimmunity.

2.3 Clinical severity of EAE is not influenced by optic nerve crush injury

Animals were injected i.p. with 10^7 T_{MBP} cells with or without concurrent optic nerve crash injury. The clinical course of the rats injected with the T_{MBP} cells was evaluated according to the neurological paralysis scale. Each group contained 5-9 rats. As can be seen in Fig. 11, the course and degree of the EAE was not affected by whether or not the rats had been subjected to an optic nerve crash.

2.4 Survival of RGCs in non-injured nerve is not influenced by T_{MBP} injection

Animals were injected i.p. with 10^7 T_{MBP} cells or PBS. Two weeks later, 4-Di-10-Asp was applied to the optic nerves. After five days the retinas were excised and flat mounted. Labeled RGCs from five fields, in each retina were counted and their average number per mm^2 was calculated.

As can be seen in Fig. 12, there is no difference in the number of surviving RGCs between rats injected with anti-MBP T-cells and control rats.

3. Summary and Conclusion

The above results demonstrate that activated T-cells accumulated at an injured site of the CNS. Furthermore, the results also demonstrate that the accumulation of T-cells at the site of injury is a non-specific process, namely T-cells which can accumulate at the site of injury include both such which are specific to antigens found at the site of injury as well as T-cell specific for antigens which are not found at the site of injury.

Furthermore, the above studies demonstrate the beneficial effect of T-cells specific for antigens found at the site of injury. More specifically,

the administration of functionally autoimmune anti-MBP T-cells (T_{MBP}), rather than aggravating the damage, actually led to a significant degree of protection from secondary degeneration. In other words, enhancing T-cell autoimmunity to a component of CNS antigen is beneficial in limiting the spread of damage.

Thus, the present findings suggest that secondary degeneration can be inhibited by the passive transfer into the individual of T-cells specific with the antigen which is found at the site of injury. In addition, the study also suggests that the same benefit effect can be obtained by inducing autoimmunity in individuals directed against antigens found at the site of CNS injury, will also help to achieve the same beneficial therapeutic effect. Thus, another treatment paradigm suggested by these findings is the induction of autoimmune reaction by administering the individual with an immunogenic antigen or immunogenic epitope of an antigen found exclusive within the CNS, as another potential therapeutic paradigm for avoiding secondary CNS degeneration following injury.

CLAIMS:

1. A method for therapeutic treatment of an individual, comprising administering to the individual an effective amount of T-cells specific for an antigen not present or present in the individual's circulation only in small quantities.

2. A method according to Claim 1, wherein the T-cells are autologous.

3. A method according to Claim 1 or 2, wherein the administration of said T-cells does not cause an autoimmune disease.

4. A method according to any one of Claims 1-3, wherein the T-cells are specific for a non-self or a CNS-specific antigen.

5. A method according to any one of Claims 1-4, for the treatment of CNS damage.

6. A method for therapeutic treatment of an individual comprising withdrawing T-cells from the individual, exposing the T-cells *in vitro* to an antigen not present or present in the circulation only in small quantities, for expansion of T-cells specific for said antigen, and then inoculating the resulting T-cell population to the individual.

7. A method according to Claim 6, wherein the T-cells are specific for a non-self or a CNS-specific antigen.

8. A pharmaceutical composition comprising, as an active ingredient, an effective amount of T-cells specific for an antigen not present or present in the circulation of a treated individual only in small quantities.

9. A pharmaceutical composition according to Claim 8, wherein said T-cells do not cause an autoimmune disease when administered to the individual.

10. A pharmaceutical composition according to Claim 8 or 9, for the treatment of CNS damage.

11. A pharmaceutical composition according to any one of Claims 8-10, prepared for the treatment of a specific individual, wherein the T-cells are autologous T-cells.

12. A method for treating an individual, comprising administering to the individual an effective amount of a CNS-specific antigen.

13. A method according to Claim 12, for the treatment of CNS damage.

14. A pharmaceutical composition comprising an effective amount of a CNS-specific antigen.

15. A pharmaceutical composition according to Claim 14, for the treatment of CNS damage.

16. A method for the preparation of a pharmaceutical composition comprising withdrawing T-cells from the blood and exposing the T-cells *in vitro* to an antigen not present or present in the circulation only in small quantities, for expansion of T-cells specific for said antigen, and then inoculating the resulting T-cell population to the individual.

17. A method according to Claim 16, for preparation of a pharmaceutical composition for the treatment of a specific individual, wherein said T-cells are autologous T-cells.

18. A method according to Claim 17, wherein the withdrawn T-cells are exposed to a non-self or CNS-specific antigens.

19. A method for the delivery of a substance to a site of damage in an individual's central nervous system (CNS), comprising activating T-cells which either carry or can produce said substance, and allowing said cells to migrate within the individual's body to reach said site.

20. A method according to Claim 19, comprising treating T-cells *in vitro* so as to cause them to either carry or produce said substance, and then activating said T-cells.

21. A method according to Claim 20, comprising loading T-cells with said substance.

22. A method according to Claim 20, comprising treating the T-cells by inserting into the cells a DNA sequence which either encodes said substance, encodes an enzyme which catalyzes said substance, or encodes a regulatory product the production of which brings to expression of said substance in the T-cells, the DNA sequence being under expression control such that they are either expressed continuously or induced to express as a result of exposure of the T-cells to a microenvironment of a kind present at the site of DNA damage.

23. A method according to any one of Claims 19-22, wherein the T-cells are autologous T-cells.

24. A method according to Claim 23, wherein said T-cells are specific for an antigen not present or present in the individual's circulation only in small quantities.

25. A method according to Claim 24, wherein the T-cells are specific for a CNS-specific antigen or for a non-self antigen.

26. A composition for the delivery of a substance to site of damage in an individual's CNS, comprising, as an active ingredient, activated T-cells which either carry or can produce said substance.

27. A composition according to Claim 26, wherein said T-cells contain an inserted DNA sequence which either encodes said substance, encodes an enzyme which catalyzes said substance, or encodes a regulatory product the production of which brings to expression of said substance in the T-cells, the DNA sequence being under expression control such that they are either expressed continuously or induced to express as a result of exposure of the T-cells to a microenvironment of a kind present at the site of DNA damage.

28. A composition according to Claim 26 or 27, for the treatment of a specific individual, wherein the T-cells are autologous T-cells.

29. A composition according to Claim 28, wherein the T-cells are specific for an antigen which is not present or present in the individual's circulation only in small quantities.

30. A composition according to Claim 29, wherein the administration of said T-cells to the individual does not cause an autoimmune disease.

31. A composition according to Claim 29 or 30, wherein said T-cells are specific for a CNS-specific antigen or for a non-self antigen.

32. A method for diagnosis of damage in the CNS of an individual, comprising inoculating the individual with activated T-cells carrying a detectable marker substance, allowing time for migration of the T-cells to a site of damage in the CNS and then detecting the presence and location of the marker substance in the individual's CNS.

33. A method according to Claim 32, wherein the T-cells are autologous T-cells.

34. A method according to Claim 32, wherein the T-cells are specific for a CNS-specific antigen.

35. A composition for use in diagnosing damage in the CNS of an individual, comprising activated T-cells carrying a detectable marker substance.

36. A composition according to Claim 35, for diagnosing of CNS damage in a specific individual, wherein the T-cells are autologous.

37. A composition according to Claim 36, wherein the T-cells are specific for a CNS-specific antigen or for a non-self antigen.

38. A method for the treatment of damage in an individual's CNS comprising inoculating the individual with activated T-cells carrying or producing a substance in need at a site of CNS damage to ameliorate the damage or symptoms associated therewith.

39. A method according to Claim 38, wherein the T-cells are autologous T-cells.

40. A method according to Claim 39, wherein the T-cells are specific for CNS-specific antigens or non-self antigens.

41. A composition for the treatment of damage in an individual's CNS, comprising, as an active ingredient, activated T-cells carrying or producing a substance in need at a site of CNS damage.

42. A composition according to Claim 41, wherein the T-cells are autologous T-cells.

43. A composition according to Claim 42, wherein the T-cells are specific for CNS-specific antigens or non-self antigens.

44. T–cells carrying or capable of producing a substance in need at a site of CNS damage, said substance being a substance which is not naturally carried or produced by the T-cells.

45. T-cells according to Claim 44, being autologous T-cells derived from the individual to be treated.

46. T-cells according to Claim 45, being specific for CNS-specific antigens or non-self antigens.

47. Cells according to any one of Claims 44-46, which have been loaded *in vitro* by said substance.

48. Cells according to any one of Claims 44-47, carrying an introduced DNA sequence which either encodes said substance, encodes an enzyme which catalyzes said substance, or encodes a regulatory product the production of which brings to expression of said substance in the T-cells, the DNA sequence being under expression control such that it is either expressed continuously or induced to expression as a result of exposure of the T-cells to a micro environment of a kind present at the site of DNA damage.

49. A pharmaceutical composition for the treatment of a human condition or disease, comprising as an active ingredient, T-cells specific for a CNS-specific antigen.

50. A pharmaceutical composition according to Claim 49, comprising also an effective amount of a CNS-specific antigen.

51. A pharmaceutical composition according to Claim 49 or 50, for countering CNS degeneration.

52. A pharmaceutical composition according to Claim 49 or 50, for inhibiting secondary degeneration, at the site of injury or lesion in the CNS.

53. A pharmaceutical composition according to any one of Claims 49-52, for the treatment of a specific individual, with the T-cells being autologous T-cells.

54. A method for the treatment of a human condition or disease comprising administering to an individual an effective amount of T-cells specific for a CNS-specific antigen.

55. A method according to Claim 54, wherein the T-cells are autologous.

56. A method according to Claim 54 or 55, comprising administering to the individual also a CNS-specific antigen.

57. A method according to any one of Claims 54-56, wherein said condition or disease is CNS degeneration.

58. A method according to Claim 57, for inhibiting progression of a secondary degeneration following injury or lesion in the CNS.

59. Use of an active ingredient being a CNS-specific autoimmune T-cell or a CNS-specific antigen, for the preparation of a pharmaceutical composition for the treatment of a human condition or disease.

60. Use according to Claim 59, wherein the human condition or disease is central nervous system degeneration.

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